The effects of settlement and anaesthesia on oxygen consumption in the New Zealand spotty (*Notolabrus celidotus*) measured in an automated flow-through respirometry system.

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Abstract

Oxygen consumption was measured in spotty (*Notolabrus celidotus*) over the 24 hours following anaesthesia. Prior to anaesthesia, respiration rate declined over the initial six to nine hours following placement of the animals in the respirometers to settle at a resting metabolic rate of 1.9 ± 0.6 $mM.kg^{-1}.h^{-1}$ (1SD). This value is at the low end of "average" respiration rates for teleost fishes, at similar temperatures. Respiration rates following anaesthesia with three different anaesthetics, with dosages being equipotent for rapid induction (100ppm MS222, 60ppm AQUI-S™, 10ppm metomidate), were anaesthetic dependent. MS222 and AQUI-S treated fishes recovered quickly and showed a marked and synchronised elevation in oxygen consumption. Metomidate treated fish showed a prolonged and more variable recovery period. Disturbance effects as the fish were introduced to the respirometers caused a much greater increase in respiration rate than any of the anaesthetics.

Key words: anaesthesia - AQUI-S - MS222 - metomidate - oxygen consumption - respirometry.

Introduction

Observations of *in vivo* physiological effects of anaesthesia show how the whole organism reacts and give clues as to which organs and systems are affected. Anaesthetic effects consist of: 1) direct anaesthetic effects; 2) secondary effects such as hypoxia and hypercapnia caused by an anaesthesia induced decrease in ventilation; 3) tertiary effects such as acid-base effects due to hypercapnia and/or the low pH of the anaesthetic; and finally 4) human effects such as handling or even the method used to monitor changes. Diurnal fluctuations in metabolic rate and reaction to being handled can produce large increases in measured

oxygen consumption of fish in respirometers (Steffensen 2002), and automated systems which minimise human disturbances can produce the lowest measured rates (Hove & Moss 1997).

Acute respiratory responses of fish to chemical inhalation anaesthesia have been measured in a number of studies. Commonly observed initial changes include hyperventilation and an increase in oxygen consumption (McFarland 1959; Summerfelt & Smith 1990), possibly as a response to the physical presence of some anaesthetics. However, during induction, hypoventilation (Houston *et al.* 1971; McFarland & Klontz 1969) and a decrease in oxygen consumption (Baudin 1932; Blahm 1961; Dixon & Milton 1978; Ross & Ross 1984) are observed as a result of anaesthesia. The reduction in oxygen consumption could be due to either a decrease in the rate of oxygen uptake and/or a decrease in metabolism. Early in recovery, hyperventilation and an increase in oxygen consumption due to oxygen debt and/or stress or increased activity often occurs (Keys & Wells 1930; Summerfelt & Smith 1990). Data on the longer term effects of anaesthetics on fish oxygen consumption are sparse. Longer term monitoring of oxygen consumption post-anaesthesia would not only elucidate the changes in oxygen consumption while recovering but would also give an estimate of recovery periods. In aquaculture fish are routinely anaesthetised to allow handling and tagging and a rapid recovery is desirable if they are to be returned to flowing water in the company of other fish. Factors other than speed of recovery may determine the choice of anaesthetic, but effects on metabolic rate should not be ignored.

These experiments utilized the Spotty (*Notolabrus celidotus*), a ubiquitous marine reef fish found in New Zealand waters. They were acclimated to a holding tube and then subjected to a brief period of anaesthesia, which involved minimal perceived disturbance. Oxygen consumption rates were measured, as an indicator of aerobic metabolism, and effects of three commonly used anaesthetics were compared.

Methods and materials

Spotties (*Notolabrus celidotus*) were caught in traps in Lyttleton Harbour, South Island, New Zealand and transported to the University of Canterbury, School of Biological Sciences. They were placed in a recirculating seawater system (10-15°C, 12 hour/12 hour artificial light/dark regime) and fed mussels (*Perna canaliculus*) two to three times per week. To avoid elevation of respiration due to digestion (specific dynamic action) fish were starved for at least 48 hours prior to respirometry experiments. Approval by the University of Canterbury Animal Ethics Committee was gained before starting any experiments.

For each determination individual spotties (mass 34.4 ± 6.3 g (2 SEMs), range 6.7 - 61.9 g, n = 30) were randomly assigned to one of three 500 mL flow through tubular respirometers constructed of opaque PVC with rubber bungs at each end. PO_2 recordings from the expelled water were then initiated (Strathkelvin Instruments, Model 781, oxygen meter and electrode, calibrated against

a second electrode). Although water temperature fluctuated from the beginning to end of the experimental series, we collected data for each of the three anaesthetics over each collection period, so that data for each group are comparable. The PO_2 of water expelled from each respirometer was measured over ten minute periods in a 40 minute cycle switching between four respirometers, using microprocessor controlled solenoids to drive the valves. With long term measurements of oxygen consumption, which are necessary to find minimal rates, the stability of the oxygen measuring device also becomes an issue. In the work reported here we used a single electrode for all four chambers, including one without an animal. Any drift, or effects of microbial oxygen demand were thus factored out of the data, which were collected on a Thurlby (DSA524) digital data storage device and were later downloaded onto a computer. The PO_2 differences between expelled water from the respirometers holding fish and from a control respirometer (identical except for the absence of a fish) were used to calculate oxygen consumption by the fish. Flow rates were controlled using a four-channel peristaltic pump (Watson Marlow) and were monitored regularly. These were varied between 5 and 50 $mL.min^{-1}$ depending on the size of the fish. As a flow-through system, PO_2 was always close to 100% saturation.

Fish were netted from holding tanks and placed into the respirometers. Oxygen consumption (MO_2) was measured over an initial 24 hour monitoring period before the spotties were anaesthetised by injecting a concentrated anaesthetic solution into the respirometers so that the final concentration caused deep anaesthesia of fish within five minutes. Three anaesthetics were tested and their final concentrations, determined prior to the experiment, were 100 ppm MS222 (Sigma Chemical Co., neutralised with 300 ppm $NaHCO_3$), 10 ppm metomidate (Wildlife Laboratories, Fort Collins, CO) and 60 ppm AQUI-S™ (active ingredient isoeugenol, kindly donated by AQUI-S New Zealand). After five minutes of exposure to the anaesthetics the respirometers were opened, refilled with clean seawater and recordings taken for another 24 hours. The few fish still responsive after five minutes of anaesthetic exposure were fully anaesthetised (stage 3, Iwama and Ackerman 1994) before being returned to the respirometers. A total of ten fish were exposed to each anaesthetic.

Two way ANOVAs (variables being anaesthetic type and time) were performed separately on the -24 to -12 hour oxygen consumption data, the data from -12 hours to immediately prior to anaesthesia, and the 24 hour post-anaesthesia data. These blocks were chosen because the initial 12 hour block should contain fish recovering from handling stress, the second 12 hour block should contain fish respiring at their routine metabolic rate (RMR) and the last 24 hour block is the treatment period. The same analysis was performed using data from both the first 12 hour block after setting up the experiment and the first 12 hours after anaesthesia, with the three anaesthetics and handling considered as the four "anaesthetic types", in an attempt to see if there was a difference between recovery post-handling and post-anaesthesia (ie. the variables were time

and anaesthetic x 3 + handling). Primary interactions were tested further using Tukey's HSD post-hoc analysis. The mean RMR of all fish was calculated from the data accumulated in the 12 hours prior to anaesthesia.

For each fish, minimum and maximum post anaesthesia metabolic rate and the time at which the fish's metabolic rate returned to within its resting value range, were recorded. ANOVA was used to determine the effect of anaesthesia on the length of time for fish to return to resting metabolic rates. ANOVA was not performed on the maxima and minima as these data would be biased by the selection criteria. Possible differences between weight specific respiration rates of different sized fish were investigated by performing a linear regression on log transformed weight and log transformed respiration rate data, and analysing the resulting line's slope to see if it was significantly different from zero. Data from the 12 hours prior to anaesthesia were used for this analysis. In all cases $P \leq 0.05$ was used to indicate a significant difference.

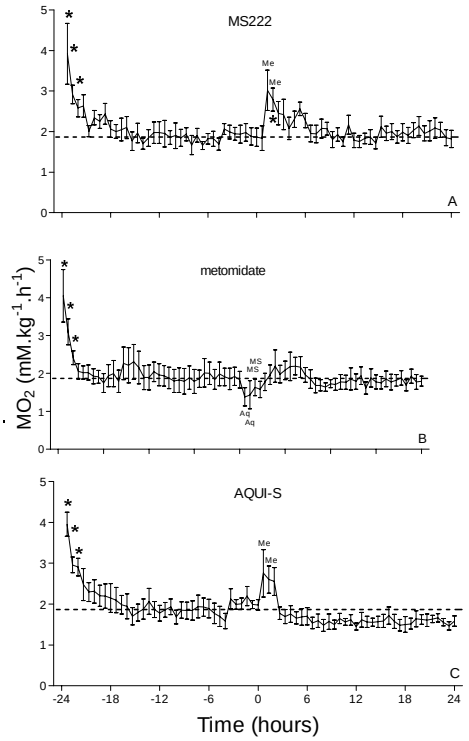

Figure 1. Graphs of oxygen consumption rates of *N. celidotus* versus time for 24 hours before and after anaesthesia with: (A) 100 ppm MS222 (neutralised with 300 ppm $NaHCO_3$), (B) 10 ppm metomidate and (C) 60 ppm AQUI-S. Data points are means ± 2SEMs. n=10 for each point. Dashed line equals mean O_2 consumption of all fish over the 12 hours pre-anaesthesia. * = significantly different from resting. MS, Me and Aq = significantly different from MS222, metomidate, AQUI-S treated fish, respectively, at the equivalent times.

Results

There was no correlation between log fish weight and log oxygen consumption (r^2 = 0.0004) and the slope of the regression line was not significantly different from zero. Therefore, raw data were not corrected for scaling effects. After placing the spotties in the respirometers there was a decrease in respiration rate with time (Figure 1). The initial elevated respiration rate was probably due to stress and activity caused by netting and exposure to air. Handling effects lasted at least three hours. There was no difference between groups for the -12 to 0 hour data, which were constant. This suggests the fish had recovered from handling and were therefore assumed to be respiring at their RMR. The mean RMR for all of the animals over this period was 1.88 ± 0.11 mM.kg^{-1}.h^{-1} (1 SEM, n = 30).

Respiration rates during recovery from anaesthesia were anaesthetic dependent. Metomidate treated fish

used less oxygen than MS222 or AQUI-S treated fish over the first one to two hours of recovery. The mean respiration rate of the MS222 treated fish significantly exceeded their mean RMR between about 1 and 1.5 hours post-anaesthesia, which suggests MS222 treated fish recovered from anaesthesia quickly and showed increased activity and/or repayment of an oxygen debt. The pattern for AQUI-S treated fish was similar but not statistically significant. Respiration rates of metomidate treated spotties were low for two to three hours and then rose above the RMR for two to three hours (not statistically significant). This lack of significance could be an artefact created by a more variable recovery time from metomidate, with peaks and troughs partially cancelling each other. For this reason maximum and minimum respiration rates for individual fish have been averaged, regardless of when they occurred (time independent).

The maximum time independent respiration rates of all groups were almost twice the RMR which suggests all fish incurred an oxygen debt and/or showed increase locomotor activity post-anaesthesia (Table 1). The maximum respiration rates of the MS222 and AQUI-S treated fish generally occurred earlier than the maximum respiration rate of the metomidate treated fish, and the time that the maximum respiration rates of the metomidate treated fish occurred tended to be more variable (data not shown). This resulted in the low and broad mean respiration peak later in the time dependent data of the metomidate treated fish (Figure 1b) and the thinner and higher time dependent respiration rate peaks of the MS222 and AQUI-S treated fish (Figure 1a&c). This suggests that increased activity occurred later following anaesthesia of the metomidate treated fish and this anaesthetic is noted for its sedative effects, as reflected in its alternative name Hypnodil. The minimum time independent respiration rates for MS222 and AQUI-S treated fish were similar to the RMR whereas the minimum time independent respiration rate of the metomidate treated fish was less than half of the RMR (Table 1). The minimum metomidate values all occurred early in the recovery, which suggests the ability of metomidate treated fish to extract oxygen was impaired, probably by anaesthesia, early in the recovery. Although MS222 and AQUI-S treated fish showed an increased $\dot{M}O_2$ during recovery, reduced oxygen consumption at the time of anaesthesia was not observed. There was no statistically significant difference between the

Table 1. Measures of recovery from anaesthesia. Time to normal is the time from initiation of recovery (removal of anaesthetic) to the start of normal respiration. Min. and Max. MR are the means of the minimum and maximum metabolic rates of each fish during recovery. Errors are ± 1 SEM and n = 10 for each value.

Comparison\Treatment	MS222	Metomidate	AQUI-S
Time to normal (h)	2.8 ± 0.3	4.7 ± 1.0	2.5 ± 0.5
Min. MR ($mM.kg^{-1}.h^{-1}$)	1.8 ± 0.3	0.9 ± 0.2	1.6 ± 0.2
Max. MR ($mM.kg^{-1}.h^{-1}$)	3.9 ± 0.4	3.5 ± 0.4	3.6 ± 0.5

anaesthetics in the mean time it took fish to return to their RMR. The recovery time of some metomidate treated fish was extended, which raised the error value of the mean (Table 1). A comparison of the handling (first 12 hour period) and anaesthesia recovery (third 12 hour period) data showed that handling caused a much greater increase in respiration rate than any of the anaesthetics.

Discussion

Measurements of metabolic rates

The lowest measured MO_2 values which we recorded are probably best described as routine metabolic rates, a designation which includes some spontaneous activity above the true basal rate (Brett & Groves 1979). The RMR for spotties (1.9 ± 0.6 $mM.kg^{-1}.hr^{-1}$ (1SD)) falls in the lower end of "average" RMR for temperate zone fish of 2.78 ± 1.06 $mM.kg^{-1}.hr^{-1}$ (1 SD, Brett & Groves 1979; Lee *et al.* 2003). Taylor's (1996) lowest recorded MO_2 value for this species in the same temperature range was 1.38 $mM.\ kg^{-1}.h^{-1}$. Spotties can occur intertidally in rock pools, especially in the size class studied here. The intertidal rock pool fishes *Acanthoclinus fuscus* and a *Forsterygion* species have routine metabolic rates of approximately 1.2 $mM.kg^{-1}.hr^{-1}$ and 1.5 $mM.kg^{-1}.hr^{-1}$ respectively for 35 g fish at 15°C (Hill *et al.* 1996). *A. fuscus* is a cryptic species with low aerobic scope, whereas spotties are relatively active reef fish, which may explain the difference in respiration rate. *F.* spp. would be intermediate between these two species with respect to activity (Hill *et al.* 1996). The generally low respiration values may reflect the cool, temperate waters around New Zealand. Fish also show diurnal patterns of activity that can confound measurements of RMR and basal metabolic rate (Steffensen 2002). One advantage of our experimental set-up is that groups of three animals were used simultaneously, each exposed to a different anaesthetic and all three should be subject to the same diurnal rhythms, disturbance and handling effects. Fish were held in opaque containers and there was no evidence of a diurnal rhythm in oxygen consumption. The opacity of the containers eliminated visual cues at the time of application of the anaesthetic and on occasions when checks were made that the system was functioning correctly, and is likely to have contributed to the lack of variability in MO_2. A disadvantage of this arrangement is that it was not possible to monitor activity. Increased activity on recovery from anaesthesia would elevate MO_2. Chinook salmon, a pelagic species, reacted vigorously when first placed in containers following anaesthesia (Hill & Forster 2004). The spotties used in these experiments recovered in an environment that they had been in for the previous 24 hours and this is a species that lives in confined spaces in the wild.

Anaesthesia with any of the anaesthetics is likely to have caused an oxygen deficit that contributed to elevated respiration rates in the first six hours of recovery, compared to the RMR. The cause of an oxygen deficit is either an impaired uptake of oxygen and/or an increased metabolic rate. Most likely the former occurs during anaesthesia, whilst additional stresses

could cause the latter. A reduction in ventilation rate and amplitude is normally observed during anaesthesia (see Bell 1987; Brown 1988; Iwama *et al.* 1989; Ryan 1992a; Ryan 1992b; Schoettger & Julin 1967) and our fish were anaesthetised to stage 3, when ventilation ceased (Iwama & Ackerman 1994). A decrease in the rate of oxygen consumption during anaesthesia has been observed in several species of fish using different anaesthetics: goldfish (*Carassius auratus*) with MS222 (Baudin, 1932); sockeye salmon (*Oncorhynchus nerka*) with MS222 (Blahm 1961); common blenny (*Blennius pholis*) with quinaldine (Dixon & Milton 1978); a variety of mullet fry with seven different anaesthetics (Durve 1975); platyfish (*Xiphophorus maculatus*) with 2-phenoxyethanol, metomidate, MS222 and quinaldine sulphate (Guo *et al.* 1994; Guo *et al.* 1995a); killifish (*Fundulus heteroclitus*) with tertiary amyl alcohol, methylparafynol, chlorobutanol and MS222 (McFarland 1960); guppies (*Poecilia reticulata*) with 2-phenoxyethanol (Teo & Chen 1993); and carp (*Cyprinus carpio*) with 2-phenoxyethanol (Yamamitsu & Itazawa 1988). A reduction in the rate of excretion of metabolic products has also been observed (Guo *et al.* 1995b) which supports the notion that metabolic rate is suppressed. However, the decrease in oxygen consumption must outweigh the decrease in metabolic rate if oxygen debt occurs. Our methodology does not allow us estimate the period over which gas exchange was impaired during anaesthesia. Following the refilling of the respirometers we could not observe the fish and estimate the period of impaired ventilation. Rates post-anaesthesia rose to c. 150% of the resting rate. Given that the period of anaesthesia was not long, though we cannot be certain when "normal" rates of oxygen consumption resumed, it is possible that the elevation of metabolism post anaesthesia was greater than the "oxygen debt". Excess post-exercise oxygen consumption can be considerably more than the calculated debt in mammals (Baker & Gleeson 1998) and fish (Scarabello *et al.* 1992) and a variety of processes contribute to elevated metabolic rates (Gaesser & Brooks 1975).

It is generally recognised that fish have relatively long recovery periods after being anaesthetised with metomidate and its analogues, etomidate and propoxate (Amend *et al.* 1982; Brown 1988; Gilderhus & Marking 1987; Mattson & Riple 1989). The anaesthetic concentrations selected were based on the rate at which anaesthesia is induced (surgical level anaesthesia within five minutes) and induction times and recovery times are not necessarily related. We used all anaesthetics at higher concentrations than are employed for routine handling of fish in aquaculture. In Chinook salmon metomidate had less effect on blood pressures in recovery than either MS222 or AQUI-S (Hill & Forster 2004). Differences between anaesthetics might be explained by their relative effects on sedation and analgesia.

Our data indicate that the oxygen debt caused by the anaesthetics is smaller than that caused by the handling during the setting up of the experiment. In a similar way cardiovascular parameters in Chinook salmon were more influenced by handling than the anaesthetics per se

(Hill & Forster 2004). Fish anaesthetics are often used to reduce stress in fish during aquaculture practices, and clearly care must be taken not to cause stress to fish as the anaesthetic is introduced. Our data also demonstrate that it is possible to introduce anaesthetics without large disturbances of metabolic rate, if the fish do not need to be physically handled.

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